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ORIGINAL ARTICLE

Leptobrachium bompu (Amphibia, Anura, Megophryidae) Discovered in Upper Medog, Tibet, China with Descriptions of its Tadpoles, Advertisement Calls and Systematic Position

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Abstract *Leptobrachium bompu* Sondhi and Ohler, 2011 was described based on a single specimen collected from Eaglenest in southern slope of Himalaya (holotype: No. KA0001/200905). In April, 2014, seven adults and three tadpoles of *Leptobrachium bompu* were collected from upper Medog, Tibet, China since most the morphological characters of the adult frogs matched the species *Leptobrachium bompu*, while the tadpoles mentioned above were determined as the same species as the adults by molecular systematics analysis. Three types of iris coloration were found in the seven adult specimens and two types of spiraculum in the three tadpoles. Advertisement calls usually had 6-8 notes and the fundamental frequency ranged from 1076 to 1466 Hz. All the specimens collected at upper Medog were clustered as one lineage with very low genetic variation and located at the base of the phylogenetic tree of the genus *Leptobrachium*.

Keywords acoustics, morphology, molecular systematics, *Leptobrachium bompu*, megophryidae, Medog, Tibet

1. Introduction

The megophryid genus *Leptobrachium* Tschudi, 1838 (type species *L. hasseltii* from Java), now containing 35 named species (Frost, 2016), is diagnosed by having a broad head and thin limbs (Dubois and Ohler, 1998). It was distributed in Southern China and northeastern India to islands of the Sunda Shelf and the Philippines (Frost, 2016). The genus was often considered to contain two subgenera, *Vibrissaphora* Liu, 1945, with adult males bearing spines on upper lip, whereas *Leptobrachium* lack this character (Ohler *et al*., 2004). As summarized by Dubois and Ohler (1998), it was a controversial issue for taxonomic recognition of *Leptobrachium* and *Vibrissaphora* as genera, subgenera, or synonyms. Molecular phylogenetic researches from Zheng *et al*. (2008) and Rao and Wilkinson (2008) discovered that *Vibrissaphora* is nested within the genus *Leptobrachium*, but they were treated as two different genera by some Chinese herpetologists in certain periods (Zhao and Kraig, 1993; Fei *et al*., 1990, 1999, 2005, 2010, 2012). Matsui *et al*. (2010) used mtDNA sequences from various populations of *Leptobrachium* (*sensu lato*), especially from Sundaland, to evaluate the taxonomic status of this genus, and then suggested two subgenera: *L. Leptobrachium* (type species *L. hasseltii*) contained species from the Philippines, Malaysia, Indonesia, Thailand, Myanmar, and India, and subgenus *L. Vibrissaphora* (type species *V. boringii*) contained species from Indochina and China.

Up to now, *Leptobrachium bompu*, described based on one specimen, i.e., holotype (No. KA0001/200905), is the only recorded species of *Leptobrachium* in Bompu (27°06'61" N, 92°40'64" E, altitude of 1 940 m), Eaglenest Wildlife Sanctuary, located at southern slope of eastern Himalayan Mountains (Sondhi and Ohler,

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2011). In 1970s, Huang Yongzhao collected one tadpole of *Leptobrachium* in Beibung of upper Medog, but there were not additional tadpoles or adults of this genus were found or collected or reported since the 1970's from upper Medog. In 2014, we surveyed upper Medog again and collected three tadpoles, and seven adults with advertisement calls records in two different localities, and these adult specimens were identified as *L. bompu* based on their external morphological characters, having black bands on the limbs, hands, feet, digits and upper lip, irregular dark markings on the dorsal surface, and distinctly wrinkled skin; while the tadpoles were determined as the same species as those adults based on molecular systematics analysis. Here we report our survey results on this species, including tadpoles, adults, advertisement calls, and systematic position.

2. Materials and Methods

2.1 Collection and measurements of specimens Field surveys were conducted in Upper Medog, Tibet, China during April 19–23, 2014. Seven adult males and three tadpoles were collected at Buqiong Lake (29°15'16.30" N, 95°13'28.80" E; altitude of 1 455 m) and Gelin Village (29°13'28.20" N, 95°11'08.50" E; altitude of 1 600 m) by Jianping Jiang and Li Ding (Figure 1).

All measurements of adults were carried out with sliding calipers to the nearest 0.1 mm following Sondhi and Ohler (2011) for the purpose of consistency. The list of measurements (Table 3) and abbreviations used are as follows:

Body SVL, snout-vent length.

Head HL, head length (from posterior corner of mandible to tip of snout); HW, head width, at the angle of jaws; SL, distance from anterior corner of eye to tip of snout; EN, distance from anterior corner of eye to nostril; NS, distance from nostril to tip of snout; IN, internarial distance; ED, eye diameter UEW, maximum width of upper eyelid. IAE, distance between anterior corner of eyes; IPE, distance between posterior corner of eyes; IUE, minimum distance between upper eyelids; MN, distance from posterior corner of mandible to nostril; MAE, distance from posterior corner of mandible to anterior corner of eye; MPE, distance from posterior corner of mandible to posterior corner of eye.

Fore limb FAHL, fore arm and hand length; HAL, hand length (from base of outer palmar tubercle to tip of third finger); F3L, third finger length (from the distal part to the articulation between proximal phalange and metacarpal bone of the third finger); F3W, width of third finger measured at the halfway length of the finger.

Hind limb ThiL, thigh length (from vent to knee); TL, tibia (shank) length; TW, maximum tibia (shank) width; TFL, length of tarsus and foot (from base of tarsus to tip of fourth toe); FL, foot length (from base of inner metatarsal tubercle to tip of fourth toe); T4L, fourth toe length (from the distal part to the articulation between proximal phalange and metatarsal bone of the fourth toe); T4W, width of fourth toe, measured at the halfway length of the toe; IMTL, length of inner metatarsal tubercle; ITL, inner toe length.

All tadpoles (the tadpoles were determined as the same species as those adults based on molecular systematics analysis, details in the section 2.3 molecular systematics and the section 3.4) were staged according to Gosner (1960) and measurements were carried out with sliding calipers to the nearest 0.1 mm as in Fei *et al*. (2009). The list of measurements (Table 4) and the abbreviations used are as follows: TOL, Total length; SVL, Snout-vent length; BH, Body height; BW, Body width; SS, Snout to spiraculum; MW, Mouth width; INS, internarial space; IOS, Interocular space; SND, snout-nostril distance; NED, nostril-eye distance ; TBW, Width of tail base; TL, tail length; TH, Tail height.

2.2 Call recordings and acoustic analyses Ten advertisement calls from two males were recorded using Marantz Professional Solid State Recorder PMD661 *MKII* with parameter setup 24 bit and 96 KHz at Gelin Village. Air temperatures varied between 22 and 23 °C during this period. We calculated audiospectrograms with fast-Fourier transform (FFT) of 512 points. Six properties were measured to quantify call characteristics of this species, including the fundamental frequency, call duration, inter-call interval, notes per call, note duration and inter-note interval. Furthermore, waveform and spectrogram of male advertisement calls were displayed with PRAAT software (Boersma and Weenink, 2009).

2.3 Molecular systematics

Preparation of DNA, PCR and DNA sequencing We obtained tissues from ethanol (95%) preserved specimens and extracted total genomic DNA using FOREGENE animal genomic DNA extraction kit (FOREGENE Biotech., Chengdu, China). Primers for DNA amplification are shown in Table 1. The PCR conditions followed Wang *et al.* (2009).

All PCR products were purified using E.Z.N.A®. Cycle-Pure Kit. Then they were used directly as templates for sequencing by an ABI 3730 sequencer, both directions were sequenced for every sample.

Table 1 Primers used in this study.

Target	Primer	Sequence (5'-3')	Reference
partial *12S rRNA* gene	P1	GCTTCAAACTGGGATTAGATACCCCACTAT	Kocher TD *et al.* (1989)
	P2	TGACTGCAGAGGGTGACGGGCGGTGTGT	Kocher TD *et al.* (1989)
partial *12S rRNA*, *tRNA*^{val} and partial *16S rRNA* gene	X1	AGAAATGGGCTACATTTTCT	This study
	T1	AAGTGATTACGCTACCTTCGC	This study
partial *16S rRNA* gene	P7	CGCCTGTTTACCAAAAACAT	Simon C *et al.* (1994)
	P8	CCGGTCTGAACTCAGATCACGT	Simon C *et al.* (1994)

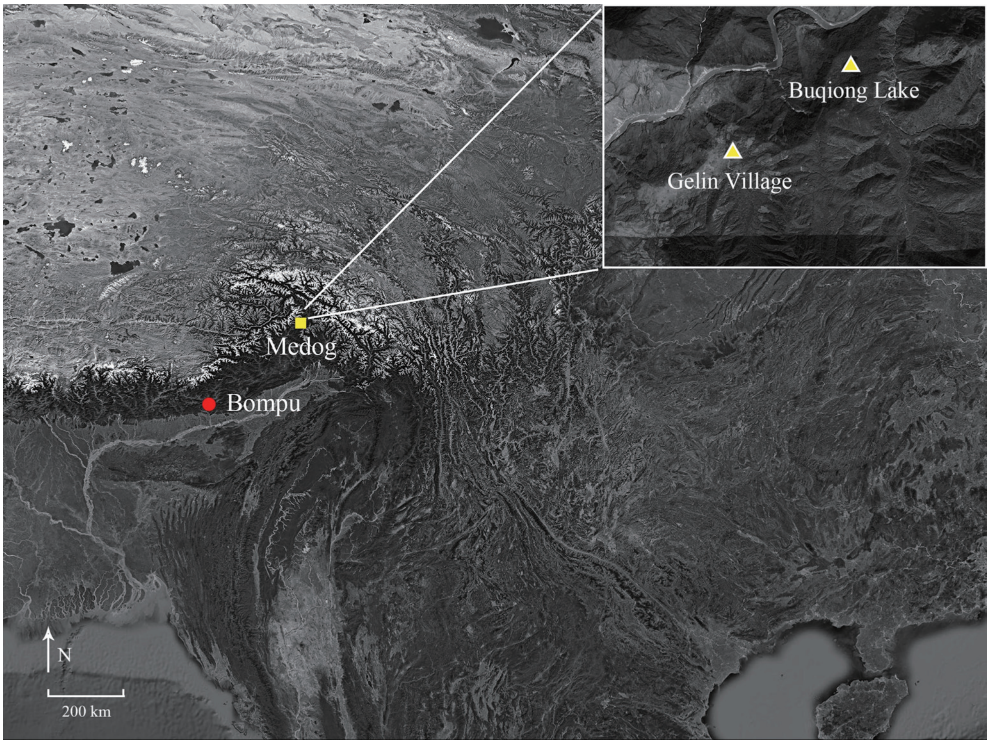

Figure 1 The localities of *Leptobrachium bompu*.

Phylogenetic analysis Thirty-nine sequences of *12S rRNA*, *tRNA*^{val}, and *16S rRNA* genes were retrieved from Frost *et al.* (2006), Fu *et al*. (2007), Matsui *et al*. (2010), Hamidy *et al*. (2011), Hamidy *et al*. (2012) and Chen *et al*. (2013), and aligned with sequences from ten new samples from Upper Medog of Tibet in China. We chose *Megophrys nasuta*, *Oreolalax rhodostigmatus*, *Scutiger boulengeri* and *Leptolalax heteropus* as outgroups. All sequences were deposited in Genbank (Accession No. see Table2).

We used Seqman (DNASTAR, Madison, Wisconsin, USA) to assemble and edit the sequences, and then aligned them using the ClustalW module in Bioedit (Hall, 1999) with default parameters. Bayesian inference (BI) and Maximum likelihood (ML) analyses as implemented in MrBayes v3.2.1 (Ronquist *et al.*, 2012) and PhyML 3.0 (Guindon *et al.*, 2010), respectively, were used to reconstruct the phylogenetic trees. We used Akaike's Information Criterion (AIC; Akaike, 1974) as implemented in jModelTest 1.0 (Posada, 2008) to select the best-fit nucleotide substitution model. The best-fitting model was GTR+I+G.

In BI, two separate runs were performed with four Markov chains. We ran Bayesian analyses for 5×10^6 generations using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm with four heated Markov chains per generation, and sampling trees every 1000 generations. We performed two Bayesian runs to ensure that independent analyses converged on similar log-likelihood scores (Leaché and Reeder, 2002). When the log-likelihood scores were found stably, a consensus tree was calculated after omitting the first 25%+ trees as burn-in. In ML, we chose the term of Best of NNI and SPR in tree topology search, which were operations in the

Table 2 Samples used for molecular phylogenetic analyses in this study together with the information on voucher, collection locality and GenBank accession numbers.

No	Species	Voucher	Locality	GenBank	References
1	*L . montanum lineage* 1	KUHE 42812	Indonesia, S. Kalimantan, Banjar, Paramasan	AB530392	I
2	*L. montanum lineage* 1	BORN 22008	Malaysia, Sabah, Tawau	AB530393	I
3	*L. montanum lineage* 2	KUHE 42813	Indonesia, C. Kalimantan, Lamandau, Belantikan	AB530395	I
4	*L. montanum lineage* 3	KUHE 17306	Malaysia, Sarawak, Kubah, Mt. Serapi	AB530397	I
5	*L. waysepuntiense*	KUHE 42805	Indonesia, Sumatra, Lampung, Kubu Perahu	AB530400	I
6	*Leptobrachium* sp.1	UTA_A53689	Indonesia, Sumatra, Jambi	AB530402	I
7	*L. gunungense*	SP 3825a	Malaysia, Sabah, Mt. Kinabalu, Sungai Carson	AB530404	I
8	*L. abbotti lineage* 3	KUHE 39295	Malaysia, Sabah, Mt. Kinabalu, Poring	AB530406	I
9	*L. abbotti lineage* 3	BORN 04B018	Malaysia, Sabah, Tawau	AB530407	I
10	*L. montanum lineage* 5	BORN 08481	Malaysia, Sabah, Crocker, Kimanis Road	AB530408	I
11	*L. lumadorum*	ABTC 76306	Philippines, Mindanao, Davao	AB530410	I
12	*L. hendricksoni*	KUHE 52150	Malaysia, Peninsula, Johor, Endau Rompin, Selai	AB530418	I
13	*L. hasseltii*	KUHE 42807	Indonesia, Sumatra, Lampung, Liwa, Kubu Perahu	AB530419	I
14	*L. nigrops*	MZB Amp1790	Indonesia, Belitung, Tanjung Pandang	AB530429	I
15	*L. kanowitense*	KUHE 42587	Malaysia, Sarawak, Kanowit	AB530430	I
16	*L. smithi*	KUHE 19281	Thailand, Loei, Phu Luang	AB530432	I
17	*Leptobrachium* sp. 4	KUHE UN tissue	Thailand, Kanchanaburi, Pilok	AB530440	I
18	*L. liui*	KUHE UN larva	China, Guanxi, Huapin	AB530441	I
19	*L. chapaense lineage* 1	KUHE 19122	Thailand, Chiang Mai, Doi Intanon	AB530444	I
20	*Leptobrachium* sp. 5	KUHE 34396	Laos, Xamneua, Phupan	AB530445	I
21	*L. echinata*	MNHN 1999.5657	Vietnam, Lao Cai, Sa Pa	AB530446	I
22	*L. hainanense*	KUHE UNL L168	China, Hainan, Wuzhi Shan	AB530447	I
23	*L. chapaense lineage* 3	MNHN 1997.5249	Vietnam, Ben En	AB530448	I
24	*L. pullum*	IEBR 2780	Vietnam, Kon Tum, Kon Plong	AB530449	I
25	*L. ngoclinhensis*	IEBR 2827	Vietnam, Kon Tum, Ngoc Linh Mountain	AB530450	I
26	*L. xanthospilum*	ROM 32177	Vietnam, Gia Lai, Tram Lap	AB530451	I
27	*L. banae*	ROM 32200	Vietnam, Gia Lai, Krong Pa	AB530452	I
28	*Leptolalax heteropus*	KUHE 15487	Malaysia, Peninsula, Perak, Larut	AB530453	I
29	*Leptobrachium* sp.	MZB Amp 11313	Indonesia, Sumatra, N. Sumatra, Martabe	AB646388	II
30	*L. abbotti lineage* 2	KUHE 53107	Malaysia, Sarawak, Bario	AB646390	II
31	*L. montanum lineage* 4	MDK 30	Indonesia, E. Kalimantan, Berau	AB646403	II
32	*L. hasseltii*	KUHE 44535	Indonesia, Java, Central Java, Mt. Ungaran	AB646408	II
33	*L. ingeri*	MZB Amp1790	Indonesia, Belitung, Tanjung Pandang	AB719252	III
34	*L. liui*	KUHE UNL L54	China, Guangxi, Huapin	AB719256	III
35	*L. ailaonicum*	MNHN 1999.5657	Vietnam, Lao Cai, Sa Pa	AB719257	III
36	*M.nasuta*	FMNH 236525	Malaysia, Sabah, Crocker,Tenom	DQ283342	IV
37	*Oreolalax rhodostigmatus*	CIB ZYCA746	China, Guizhou, Da Fang Co.	EF397248	V
38	*Scutiger boulengeri*	ROM 40445	China, Sichuan, Xiaojin Co.	EF397273	V
39	*L. guangxiense*	NHMG_T201205021	China, Guangxi, Shangsi	JX467673	VI
40	*L. bompu*	CIB LX551	China, Tibet, Medog	KU900359	This study
41	*L. bompu*	CIB LX552	China, Tibet, Medog	KU900360	This study
42	*L. bompu*	CIB LX553	China, Tibet, Medog	KU900361	This study
43	*L. bompu*	CIB LX555	China, Tibet, Medog	KU900362	This study
44	*L. bompu*	CIB LX678	China, Tibet, Medog	KU900364	This study
45	*L. bompu*	CIB LX679	China, Tibet, Medog	KU900365	This study
46	*L. bompu*	CIB LX680	China, Tibet, Medog	KU900366	This study
47	*L. bompu*	CIB LX692	China, Tibet, Medog	KY486756	This study
48	*L. bompu*	CIB LX693	China, Tibet, Medog	KY486757	This study
49	*L. bompu*	CIB LX694	China, Tibet, Medog	KY486758	This study

Note: I: Matsui *et al.* (2010b); II: Hamidy *et al.* (2011); III: Hamidy *et al.* (2012); IV: Frost *et al.* (2006); V: Fu *et al.* (2007); VI: Chen *et al.* (2011).

Table 3 Measurements of adult males of *Leptobrachium bompu* from upper Medog (mm).

Variable	7♂♂
SVL	51.8 ± 1.4 (50.0–53.5)
HL	21.9 ± 0.5 (21.3–22.6)
HW	23.6 ± 0.5 (22.9–24.6)
SL	8.7 ± 0.4 (8.0–9.1)
EN	3.9 ± 0.2 (3.6–4.1)
NS	4.6 ± 0.2 (4.4–5.0)
IN	5.2 ± 0.5 (4.7–6.1)
ED	6.1 ± 0.3 (5.5–6.6)
UEW	5.5 ± 0.2 (5.2–5.8)
IAE	8.9 ± 0.1 (8.7–9.1)
IPE	17.0 ± 0.6 (16.3–17.9)
IUE	5.9 ± 0.3 (5.4–6.4)
MN	17.9 ± 0.4 (17.2–18.4)
MAE	14.2 ± 0.6 (13.3–15.0)
MPE	7.6 ± 0.2 (7.3–7.8)
FAHL	28.7 ± 0.5 (27.8–29.5)
HAL	15.6 ± 0.3 (15.2–15.9)
F3L	10.45 ± 0.5 (9.9–11.5)
F3W	2.3 ± 0.2 (1.9–2.5)
ThiL	23.1 ± 0.8 (21.9–24.5)
TL	20.8 ± 0.6 (20.1–21.7)
TW	6.7 ± 0.5 (5.7–7.2)
TFL	31.1 ± 1.0 (29.8–32.5)
FL	21.8 ± 1.4 (19.9–23.3)
T4L	14.9 ± 0.9 (13.5–15.9)
T4W	2.1 ± 0.2 (1.8–2.2)
IMTL	3.4 ± 0.5 (2.5–3.9)
ITL	3.7 ± 0.3 (3.3–4.0)

tree searching menu and default parameters. Bootstrap support (BS) values calculated in the PhyML 3.0 (Guindon *et al.*, 2010) automatically.

3. Results

3.1 Morphological characters

Adults No adult female were found during this survey. All seven adult males collected in this work presented almost the same external morphological characters as the specimen found in Bompu at Eaglenest reported by Sondhi and Ohler (2011): no spines on upper lip in adult males; head wider than length; tympanum indistinct. Second finger shortest; finger tips swollen; several tubercles formed callous ridges beneath all fingers (Figure 2G); two prominent metacarpal tubercles present and supernumerary tubercle absent (Figure 2G). Tibia longer than 40 % of snout-vent length; toe tips swollen; webbing leaving 3 ½ phalanges of toe IV and 2 phalanges of toe V free; length of inner metatarsal tubercle almost equals first toe length; several tubercles formed callous ridges beneath all toes (Figure 2H). Back and flank covered with fine ridges forming reticulum and irregular distinct darker markings; ventrum dark purple in life but grey-black in formalin with small white spots; iris of uniform grey-blue coloration, sclera black.

There were also some differences between these two batches of specimens from upper Medog and Bompu: the specimens from Medog have a larger body size, SVL = 51.8 ± 1.4 (50.0–53.5, n = 7) (Table 3) than Bompu's (SVL = 47.0, n = 1). In addition, seven individuals presented three types of iris coloration: (1) Each of the two eyes had uniform grey-blue (Figure 2D); （2）One eye was a uniform grey-blue, another was black brown (Figure 2E); (3) Both of two eyes were black (Figure 2F).

Only males were found in this time. Their vocal sacs are not distinct externally; openings as small slits, positioned near the proximal part of the tongue, surrounded by skin folds. Nuptial spines absent.

Tadpoles (Figure 3) Three tadpoles were at stages from 35, 36 and 37, respectively (Table 4). They were collected together with the adult males. Two had the spiraculum on the left side of the body, as the Acosmanura type (Starrett, 1973) (Figure 3A), while the third one the spiraculum on the right side of the body (Figure 3B). Preserved in alcohol, dorsal part of body brown yellowish with thick gray spots while ventrum grey white, but tail thick gray with large brown yellowish spots; tail end rounded; both lower and upper labium with big papillae, a few submarginal papillae occur at corner of the mouth, upper labium with a medial gap of about one papillae width; lower tail fin lighter than upper one; the keratodont formula of the three tadpoles were different, I:4-4/I:4-4 (stage 35), I:5-5/I:4-4 (stage 36) and I:5-5/I:5-5 (stage 37) (Figure 3C). The measurements based on the three tadpoles were summarized in Table 4.

3.2 Call characteristics The toads continued advertisement calls at night, but occasionally called at sunset. Sounds were similar to those reported from *L. bompu* by Sondhi and Ohler (2011). The advertisement calls of the males were loud, a croaking "*kek kek kek kek*" calls with a series of identical repeated notes (range: 7.5 ± 0.53; Figure 4). Call duration, inter-call interval, note duration and inter-note interval were 3169.6 ± 1526.5 ms, 3879.1 ± 2318.2 ms, 127.4 ± 26.2 ms and 342.6 ± 46.7 ms (n = 82), respectively. Moreover, there was only one harmonic band in a note with the fundamental frequency ranged from 1076 to 1466 Hz (1258.6 ± 49.5 Hz, n = 82) (Figure 4).

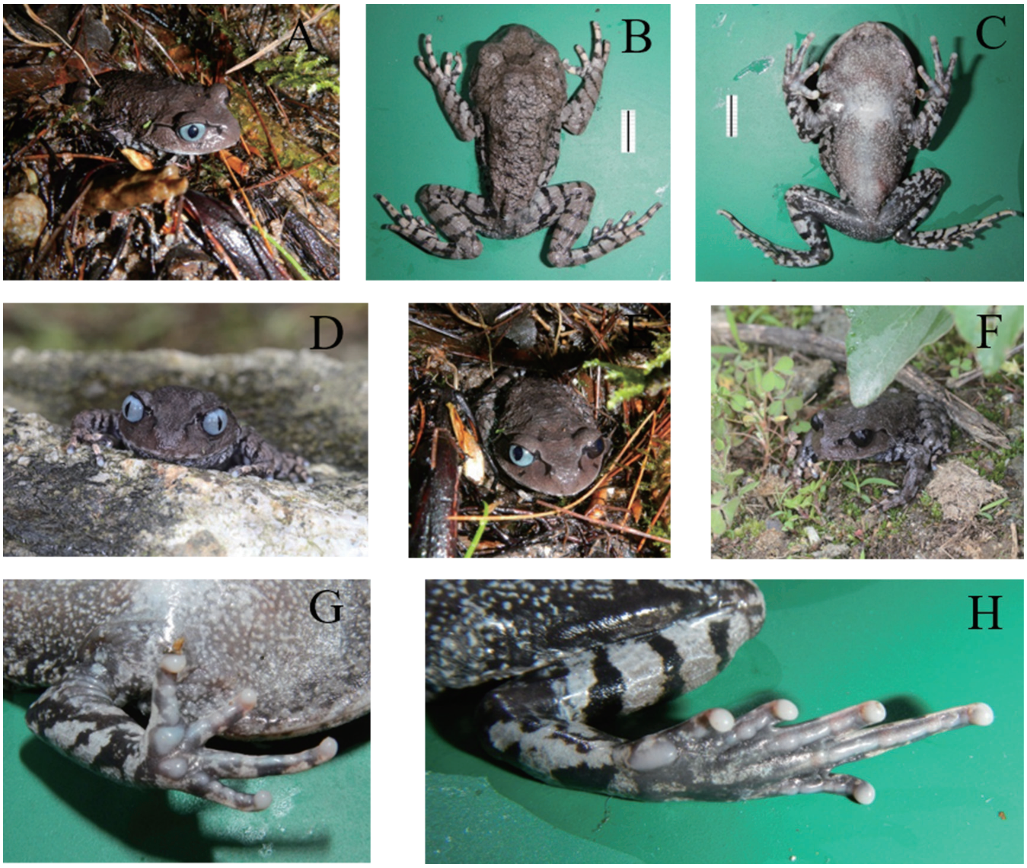

Figure 2 Adult male of *Leptobrachium bompu* from the upper Medog in southeastern Tibet of China. A: in life; B: Dorsal view; C: Ventral view of specimen 140431. Bar = 10 mm; D: both eyes were blue; E: one was blue and anther was black; F: both eyes were black; G: hand, showing metarcarpal tubercles, subarticular tubercles, and supernumerary tubercles; H: foot, showing subarticular tubercles.

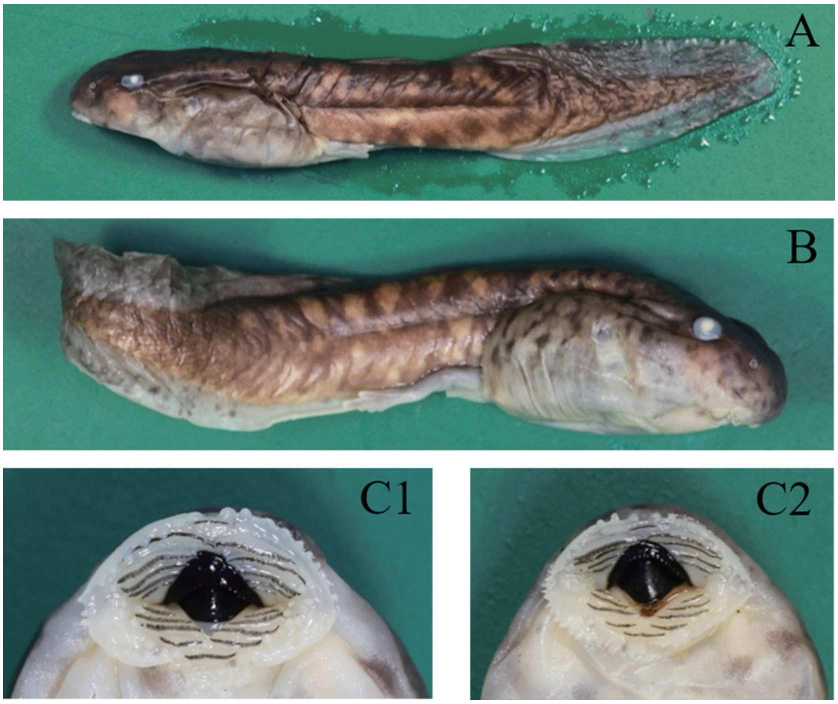

Figure 3 Tadpoles of *Leptobrachium bompu* from upper Medog in southeastern Tibet of China. A: Spiraculum on the left of body; B: Spiraculum on the right of body; C: keratodont formula (C1: stage 37, C2: stage 35).

3.3 Ecology, Behavior and Habitats The toads were observed on April 19–23, 2014 after sundown (1830 to 2200 h). They were found at two sites, Gelin Village and Buqiong Lake. The weather was cloudy and it was raining during the survey. At Gelin Village, the advertisement calls were heard in the upper reaches of a small stream (less than one meter width) in a broad-leaved forest (Figure 5A). At Buqiong Lake, advertisement calls were documented in similar living conditions (Figure 5B). The tadpoles (Figure 3) were found in the lower reaches of the two streams at Gelin and Buqiong on the same day as adults.

3.4 Systematics position of *L. bompu* in the genus *Leptobrachium* The aligned mitochondrial dataset consisted of 2007 bps, in which 1040 sites were variable, and 805 potentially phylogenetically informative. The BI and ML phylogenetic trees showed the same topologies (Figure 6), indicating *Leptobrachium* (*sensu lato*) was divided into three clades: Clade A, Clade B and Clade C. All samples of *L. bompu* clustered together and formed the Clade C（BPP = 100%, MLBS = 100%), indicating the three tadpoles and the seven adults are the same species. The evolutionary relationship of Clade A（BPP = 100%, MLBS = 100%) and Clade B（BPP = 100%, MLBS = 96%) were similar to Zheng *et al*. (2008), Matsui *et al*. (2010), Hamidy *et al*. (2011) and Chen *et al*. (2013).

4. Discussion

Leptobrachium bompu has been known only from Bompu (27°06'61" N, 92°40'64" E; altitude of 1 940 m), at the northernmost distribution limit of the genus *Leptobrachium* (Frost 2016). The new discovery of *L. bompu* in upper Medog moved the northern limit to northern latitude 29°15'16.30'', 338 km (straight line distance) from Bompu (Figure 1). In addition, Bompu is far from other species distribution sites, these two new sites and their basic biological information will provide important clues for further understanding of the distribution pattern and evolutionary history of this species and the genus *Leptobrachium*. This discovery demonstrates that the area’s herpetofauna is not well studied and new findings are still expected.

We heard advertisement calls of *L. bompu* at Buqiong Lake (29°15'16.30" N, 95°13'28.80" E; altitude of 1 455 m) and Gelin Village (29°13'28.20" N, 95°11'08.50" E; altitude of 1 600 m) during April 19–23, 2014. It was almost one month earlier than the report in Bompu by Sondhi and Ohler (2011), inferring that this species probably started breeding at least in the second half of April. But we did not find any females, eggs, or hatchlings of the species.

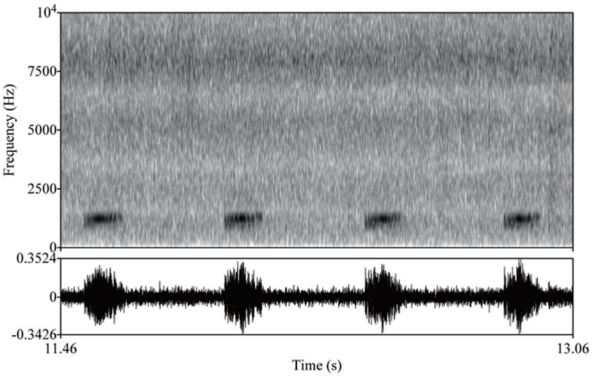

Figure 4 Sound spectrogram and waveform of four notes within a call of *Leptobrachium bompu*.

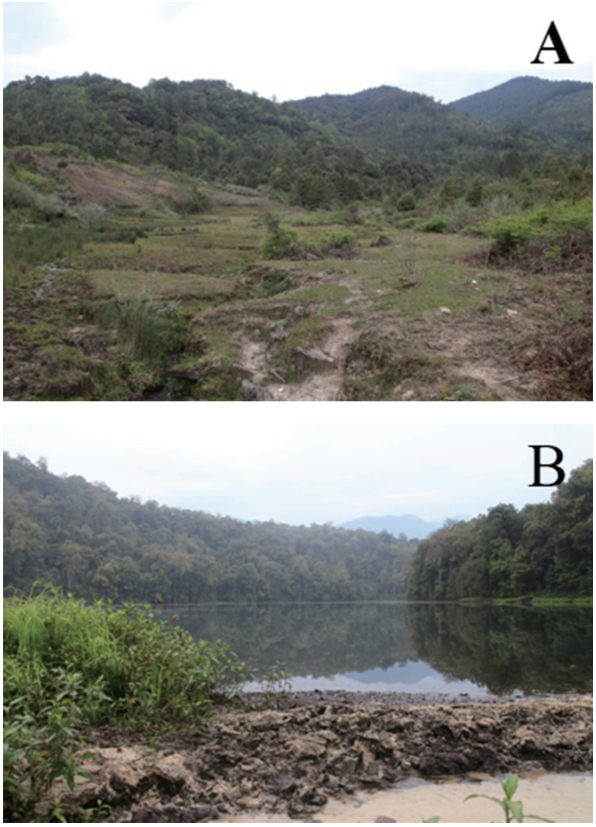

Figure 5 Habitats of *Leptobrachium bompu* in upper Medog of Tibet of China. A: Gelin Village; B: Buqiong Lake.

Matsui *et al*. (2010) and Sondhi and Ohler (2011) summarized the iris coloration of *Leptobrachium*. Though a few of new species were discovered after these two papers, in general, there were two predominate color types of iris, one type is the black or nearly black iris (present in twelve species: *L. montanum*, *L. gunungense*, *L. abbotti*, *L. hasseltii*, *L. chapaense*, *L. mouhoti*,

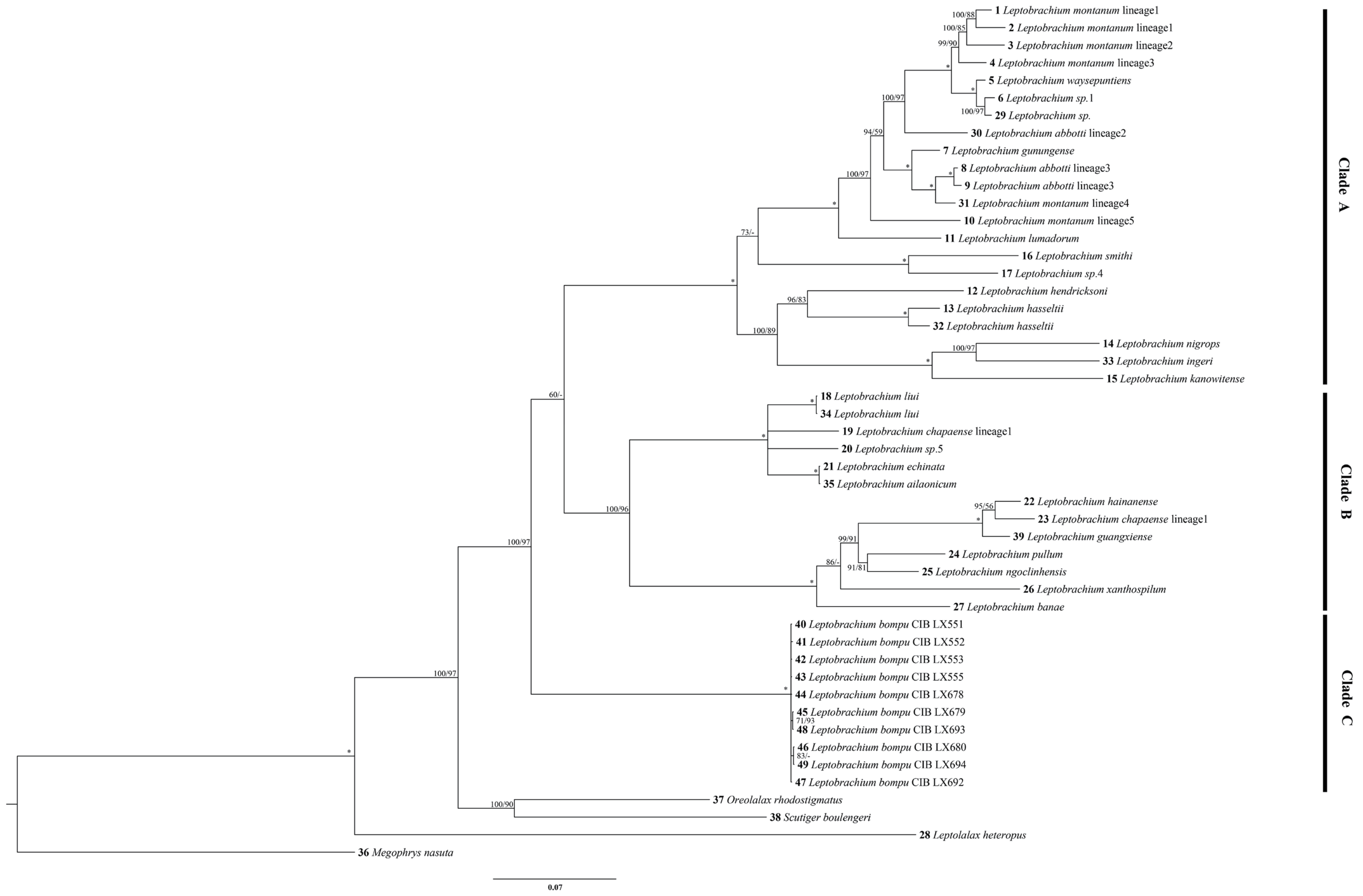
Clade A
Clade B
Clade C
1 Leptobrachium montanum lineage1
2 Leptobrachium montanum lineage1
3 Leptobrachium montanum lineage2
4 Leptobrachium montanum lineage3
5 Leptobrachium waysepuntiens
6 Leptobrachium sp.1
29 Leptobrachium sp.
30 Leptobrachium abbotti lineage2
7 Leptobrachium gunungense
8 Leptobrachium abbotti lineage3
9 Leptobrachium abbotti lineage3
31 Leptobrachium montanum lineage4
10 Leptobrachium montanum lineage5
11 Leptobrachium lumadorum
16 Leptobrachium smithi
17 Leptobrachium sp.4
12 Leptobrachium hendricksoni
13 Leptobrachium hasseltii
32 Leptobrachium hasseltii
14 Leptobrachium nigrops
33 Leptobrachium ingeri
15 Leptobrachium kanowitense
18 Leptobrachium liui
34 Leptobrachium liui
19 Leptobrachium chapaense lineage1
20 Leptobrachium sp.5
21 Leptobrachium echinata
35 Leptobrachium ailaonicum
22 Leptobrachium hainanense
23 Leptobrachium chapaense lineage1
39 Leptobrachium guangxiense
24 Leptobrachium pullum
25 Leptobrachium ngoclinhensis
26 Leptobrachium xanthospilum
27 Leptobrachium banae
40 Leptobrachium bompu CIB LX551
41 Leptobrachium bompu CIB LX552
42 Leptobrachium bompu CIB LX553
43 Leptobrachium bompu CIB LX555
44 Leptobrachium bompu CIB LX678
45 Leptobrachium bompu CIB LX679
48 Leptobrachium bompu CIB LX693
46 Leptobrachium bompu CIB LX680
49 Leptobrachium bompu CIB LX694
47 Leptobrachium bompu CIB LX692
37 Oreolalax rhodostigmatus
38 Scutiger boulengeri
28 Leptolalax heteropus
36 Megophrys nasuta
100/88
100/85
99/90
100/97
100/97
94/59
100/97
73/-
96/83
100/89
100/97
60/-
95/56
99/91
86/-
91/81
100/96
100/97
71/93
83/-
100/97
100/90
0.07

Figure 6 Bayesian phylogram of 2007 bp of *12S rRNA*, *tRNA*^{val} and *16S rRNA* mitochondrial genes for *Leptobrachium bompu* and related species. Sample numbers are included in Table 4. Numbers above branches represent bootstrap supports (BS) for Bayesian posterior probability (BPP) and maximum likelihood (MLBS). Symbol (*) indicates nodes with full bootstrap supports for Bayesian posterior probabilities (PP = 100%) and ML inferences (100%). Symbol (-) indicates node values are less than 50%.

Table 4 Measurements of tadpoles of *Leptobrachium bompu* from upper Medog (mm).

Stage	TOL	SVL	BH	BW	SS	INS	IOS	SND	NED	MW	TL	TH	WBD
35	69.1	23.7	10.4	11.8	13.8	5.2	8	2.5	3.8	6.6	45.4	12	5.6
36	72.1	26.3	14.1	13.9	14.5	6	9.2	2.1	4.3	7.3	45.8	15.7	5.8
37	83.1	25.5	13.8	15.7	16	5.8	9.3	2	4.7	7.2	57.6	15.4	7.1

L. nigrops, *L. ingeri*, *L. kanowitense*, *L. kantonishikawai*, *L. angyanorum and L. tagbanorum*), and the other type was a light-colored (ranging from white through sky-blue to lime green) upper iris (present in twelve species: *L. liui*, *L. jiulongshanensis*, *L. leishanensis*, *L. boringii*, *L. ailaonicum*, *L. echinata*, *L. promustache*, *L. hainanense*, *L. ngoclinhensis*, *L. banae*, *L. xanthospilum* and *L. leucops*). However, variations occurred, the upper part of the iris is yellow, pale yellow, orange, scarlet, or red in *L. hendricksoni*, *L. xanthops*, *L. smithi*, *L. mouhoti*, and *L. pullum*, respectively. Furthermore, in *L. hendricksoni*, the iris color varies from having color only on the upper part to be totally orange (in one juvenile); *L. rakhinensis* has bicolored red and black with the upper half red (Wogan, 2012); *L. waysepunctiense* has totally blue iris in adults, but is grey in juvenile. Sondhi and Ohler (2011) found that *L. bompu* from Bompu also had a totally blue iris in adult male and lacked the black reticulations in the blue eyes present in *L. waysepuntiense*. Interestingly in our observation of *L. bompu*, there are three types of iris coloration present, indicating that this species has highly variable iris coloration. Hence, when we use iris color to identify species in the genus *Leptobrachium*, we also should consider the possible variation.

The phylogenetic results strongly supported the monophyly of the three tadpoles and seven adults of *L. bompu*, indicating they are the same species, which is distinct from other species of *Leptobrachium*.

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